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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,274,186,** on June 10, 1999, by **MDS INC.**, assignee of Lisa Cousin and Bruce Thomson, for "Analysis Technique, Incorporating Selectively Induced Collision Dissociation and Subtraction of Spectra".

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ABSTRACT OF THE DISCLOSURE

A method of and apparatus for analyzing a substance takes a stream of ions in said substance and supplies the ions to a collision cell including a quadrupole rod set for guiding the ions and a buffer gas. An RF voltage is applied to the quadrupole rod set to guide ions. An additional alternating current signal is applied to the quadrupole rod set at a frequency selected to cause resonance excitation of the secular frequency of a desired ion, whereby said desired ions are excited and undergo collision with the buffer gas causing fragmentation. The invention then provides modulation of the alternating current signal applied in step (4) whereby periods in which said alternating current signal is applied alternate with periods in which said alternating signal is not applied. The ion spectrum after fragmentation is collected to generate one set of data for one spectrum, representative of the ion spectrum when the alternating current signal is applied, and a another set of data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied. These two spectra can be subtracted to give a spectrum indicative of the effect of fragmentation.

Title: ANALYSIS TECHNIQUE, INCORPORATING SELECTIVELY INDUCED COLLISION DISSOCIATION AND SUBTRACTION OF SPECTRA

FIELD OF THE INVENTION

This invention relates mass spectrometers, and more particularly is concerned with collision-induced dissociation (CID) in a tandem mass spectrometer. The invention is particularly intended to enable multiple stages of fragmentation, and hence mass analysis or spectroscopy, to be effected in a collision cell.

BACKGROUND OF THE INVENTION

Radio frequency (RF) only multipole spectrometers, more particularly quadrupole spectrometers, are widely applied in mass spectrometry and nuclear physics, due to their ability to transport ions with minimal losses. During such transportation of the ions, the initial ion positions and velocities change, but the total phase space volume occupied by the ion beam remains constant (see Dawson, Quadrupole mass spectrometry and its applications). However, if a buffer gas is introduced into the ion guide, a dissipative process occurs, due to ion molecule collisions, and this enables an ion beam to be focused onto the quadrupole axis after the initial velocities have been damped.

Collisional quadrupole or other multipole devices have been used as an ion guide providing an interface between an ion source and a mass spectrometer, or alternatively as a collision cell for collision-induced dissociation (CID) experiments. As a straightforward interface, collisional damping reduces the space and velocity distributions of the ions leaving the ion source, thus improving the beam quality. For CID experiments, primary ions having relatively large velocities enter the multipole and collide with buffer gas molecules, and so collision-induced dissociation takes place. The multipole helps to keep both primary ions and fragment ions, resulting from the collision-induced dissociation, close to the axis and to deliver them to the exit for further analysis. Collisions inside the multipole

spectrometer again act to reduce the space and velocity distribution of the ion beam.

Ion motion in a perfect quadrupole field is governed by Mathieu's equation (See Dawson as cited above); ions oscillate around the quadrupole axis at an appropriate fundamental frequency which is determined by their m/z and quadrupole parameters, and is independent of ion position and velocity. If the frequency of any periodic forces acting on ions coincides with the ion fundamental frequency, then resonance excitation takes place. Similar resonance excitation is widely applied in quadrupole ion trap or in ion cyclotron resonance mass spectrometers (R.E. March, R.J. Hughes, Quadrupole storage mass spectrometry, 1989, John Wiley&Sons).

These properties of spectrometers have been employed in many ways. Thus, in U.S. provisional patent application 60/046,926 filed May 16, 1997 (and related U.S. patent application 09/066,556 and Canadian patent application 2,236,199), there is disclosed a high pressure MS-MS system. This was intended to provide improvements to a conventional triple quadrupole mass spectrometer arrangement, employing two precision quadrupole mass spectrometers separated by an RF-only quadrupole which is operated as a gas collision cell. The first mass spectrometer is used to select a specific ion mass-to-charge ratio (m/z), and to transmit the selected ions into the RF-only quadrupole or collision cell. In the RF-only quadrupole collision cell, some or all of the parent ions are fragmented by collisions with the background gas, commonly argon or nitrogen, at a pressure of up to several millitorr. The fragment ions, along with any unfragmented parent ions are then transmitted into the second precision-quadrupole which is operated in a mass resolving mode. Usually, the mass resolving mode of this second spectrometer is set to scan over a specified mass range, or else to transmit selected ion fragments by peak hopping, i.e. by being rapidly adjusted to select specific ion m/z ratios in sequence. The ions transmitted through this spectrometer are detected by an ion detector. A problem with this conventional arrangement is that the two mass

resolving quadrupoles are required to operate in the high vacuum region (less than 10^{-5} torr), while the intermediate collision cell operates at a pressure up to several millitorr. That earlier invention was intended to simplify the apparatus and eliminate the necessity for separate RF-only and resolving spectrometers at the input to the apparatus. Instead, a single quadrupole is provided, operating in the RF-mode to act as a high pass filter. Additionally, this quadrupole is provided with an AC field, which can be identified as a "filtered noise field", which contains a notch in the frequency range corresponding to the mass of an ion of interest. This notch can be moved, to select and separate desired ions.

Other older proposals can be found, for example, in U.S. Patent 5,420,425 (Bier et al. and assigned to Finnigan Corporation). This relates to an ion trap mass spectrometer, for analyzing ions. It has electrodes shaped to promote an enlarged ion occupied volume. A quadrupole field is provided to trap ions within a predetermined range of mass to charge ratios. Then, the quadrupole field is changed so that trapped ions with specific masses become unstable and leave the trapping chamber in a direction orthogonal to the central axis of the chamber. The ions leaving the spectrometer are detected, to provide a signal indicative of their mass-to-charge ratios. One method that is taught in this patent is to first introduce ions within a predetermined range of mass-to-charge ratios into the chamber and subsequently change the field to select just some ions for further manipulation. The quadrupole field is then adjusted so as to be capable of trapping product ions of the remaining ions, and the remaining ions are then dissociated or reacted with a neutral gas to form those product ions. Subsequently, the quadrupole field is changed again, to remove, for detection, ions whose mass-to-charge ratios lie within the desired range, which ions are then detected.

The first technique taught above is complex, and requires a number of separate quadrupoles or the like, and the ability to move the ions sequentially through the different quadrupole sections. The technique taught in the Finnigan patent is complex and requires a number of steps.

Also, it is concerned with ion traps and not a flow quadrupole. Accordingly, it is desirable to provide one technique which, in one device, readily enables ions of a selected mass-to-charge ratio to be subject to collision-induced-dissociation (CID) or fragmentation, so that the fragments can be transported further for subsequent analysis. It is desirable to provide this in a single device, since movement of ions from one device to another inevitably leads to some losses. Similarly, the techniques of the Finnigan patent work with pulse ion sources, but attempts to use them for continuous ion flow, for instance from an electrospray ion source, will lead to inefficiencies. In this field, spectrometers are frequently used to analyze small samples, and often, high efficiency is required, if any reliable reading or measurement is to be obtained.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided a method of analyzing a substance, the method comprising the steps of:

(1) creating a stream of ions in said substance;

(2) supplying the ions to a collision cell including a quadrupole rod set for guiding the ions and a buffer gas; [

(3) applying an RF voltage to the quadrupole rod set to guide ions through the quadrupole rod set;

(4) supplying an additional alternating current signal to the quadrupole rod set at a frequency selected to cause resonance excitation of the secular frequency of a desired ion, whereby said desired ions are excited and undergo collision with the buffer gas causing fragmentation;

(5) modulating the alternating current signal applied in step (4) whereby periods in which said alternating current signal is applied alternate with periods in which said alternating signal is not applied;

(6) analyzing the ion spectrum after fragmentation and collecting one set of data for one spectrum, representative of the ion spectrum when the alternating current signal is applied and a another set of

data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied.

The alternating current signal can be applied at a frequency which is twice the secular frequency of the desired ion.

Preferably, the method includes passing the stream of ions through a first mass analyzer to select a precursor ion of interest, and passing the precursor ion into the collision cell.

More preferably, the method includes providing a potential difference between the first mass analyzer and the collision cell, to accelerate the precursor ion into the collision cell, whereby the precursor ions gain sufficient velocity to collide with the buffer gas to cause fragmentation, and wherein step (4) comprises applying an alternating current signal to excite a fragment of the precursor ion, said fragment comprising the desired ion.

The method can include applying a second alternating current signal to the quadrupole rod set, to excite a fragment ion resulting from resonance excitation of said desired ion, thereby to generate secondary fragment ions and wherein step (5) comprises modulating the second alternating current signal. It will be appreciated that it may be possible to apply a number of different excitation signals to cause fragmentation of fragments from the previous step.

Advantageously, the method includes subtracting one spectrum from the other spectrum to obtain a subtracted spectrum.

Another aspect of the present invention provides an apparatus, for analyzing a substance by resonance excitation of selected ions and selective collision-induced dissociation, the apparatus comprising:

an ion source for generating a stream of ions;

a collision cell, including a quadrupole ion guide, for receiving a stream of precursor ions and provided with a buffer gas, for collision-induced dissociation between the parent ions and the buffer gas;

a power supply connected to the quadrupole rod set for generating an RF field in the quadrupole rod set for guiding ions and for applying an additional alternating current field at a frequency selected to

excite a desired ion;

a modulation means connected to the power supply, for modulating the alternating current signal, whereby periods in which said alternating current signal are applied alternate with periods in which the alternating current signal is not applied.

Preferably, the apparatus additionally includes a detector for detecting fragment ions exiting the collision cell, a switch connected to the detector, two data storage devices connected to the switch, and a connection between the modulation control unit and the switch, whereby the switch switches detected data for periods when the alternating current signal is applied to one data storage device and collected data for periods when the alternating current signal is not applied to the other storage device.

To enable a second excitation step to be effected, the apparatus can include a second power supply connected to the quadrupole rod set, a second modulation unit connected to the second power supply and also to the switch, before applying a second alternating current signal, for excitation of a second ion.

Preferably the apparatus includes a first mass analysis section for selecting a parent ion and a final mass analysis section, including the detector, for analyzing fragment ions from the collision cell.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

For a better understanding of the present invention and to show more clearly how it may be carried into effect, reference will now be made, by way of example, to the accompanying drawings in which:

Figure 1 is a schematic of a first embodiment of an apparatus in accordance with the present invention;

Figure 2 is a schematic of an apparatus in accordance with a second embodiment of the present invention;

Figures 3a-3e are mass spectra showing analysis of bosentan and fragments thereof;

Figures 4a and 4b are detailed graphical spectra of fragments

obtained from fragmentation of a fragment of mass 202 of bosentan;

Figures 5a, 5b and 5c are spectra showing fragmentation of taxol;

Figures 6a-6f are mass spectra showing various fragmentation schemes for reserpine; and

Figures 7a-7c and Figures 8a-8c are mass spectra showing other fragmentation schemes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A description is first given of the apparatus in Figures 1 and 2. The two apparatus are largely similar, except for the final mass analysis stage. Figure 1 shows a variant with a quadrupole rod set and detector as the final mass analysis stage, while this is effected by a time-of-flight section in Figure 2.

Referring first to Figure 1, the first variant of the apparatus is indicated at 10. In known manner, the apparatus 10 includes a first quadrupole rod set generally indicated as Q0. Q0 is intended to collimate ions received from an electrospray source or the like. In known manner, upstream of Q0, there would be an ion inlet, skimmers, intermediate pressure stages and the like, all intended to remove gas and reduce pressure down to that required for mass analysis (these elements and associated pumps are not shown). Q0 collimates the ion beam and further serves to reduce gas pressure.

Ions from Q0 pass through an interquad aperture 12 into a quadrupole rod set Q1, which functions as a first mass analysis section. In known manner, Q1 is supplied with resolving RF and DC voltages. These can be conventional and the power supplies are not shown.

From Q1, the ions pass through into a collision cell housed in a chamber generally indicated 14. The collision cell includes a quadrupole rod set Q2. The chamber 14 includes, at either end, an inlet interquad aperture 16 and an exit interquad aperture 18.

The ions then pass into a final quadrupole Q3. Q3 again

would be provided with resolving RF and DC voltages, and the power supply for these is not shown. Finally, the ions pass through to a detector 20.

In known manner, appropriate DC potentials would be provided between the different quadrupole sections Q0, Q1, Q2 and Q3 and also appropriate potentials on the interquad apertures 12, 16, 18, together with an appropriate potential drop to the detector 20. These various potentials ensure movement of ions axially, from left to right in Figure 1, in known manner.

Quadrupoles Q1, Q3 would be maintained at a low pressure of 10^{-5} torr, as is known for mass resolving quadrupoles. Chamber 14 is operated as a collision cell and would be provided with a suitable collision gas (source not shown). Typically, it is operated at a pressure in the range 0.5-20 mTorr. A suitable collision gas is nitrogen.

In accordance with the present invention, a first MS step is effected in Q1 and would be designated as MS1. This selects a parent or precursor ion, which then passes into the rod set Q2 of the collision cell. To effect a second MS step, an excitation source 22 for rod set Q2 is indicated. Practically, this excitation source will simply be a potential drop between Q1 and Q2. Q2 is provided with an excitation frequency of, for example, 1,000 volts at 2 MHz. This excitation frequency would be provided in a quadrupolar manner, i.e. with the cos ωt provided to one opposite pair of rods in the quadrupole rod set Q2, and - cos ωt provided to the other, diagonally opposite pair of rods of the rod set Q2.

The potential drop into rod set Q2 accelerates ions and causes them to collide with a collision gas, causing fragmentation. Such a fragmentation step is known in the art.

Now, in accordance with the present invention, the rod set Q2 is further excited to effect either one or a multiple steps of excitation.

Firstly, a further excitation step MS3 is effected by an excitation source 24 provided with a modulation control unit 26, whose function is explained below. To effect a fourth fragmentation step, a second

power supply 28 is provided, connected to a second modulation control unit 30. Each of the power supplies 24, 28 can provide a similar signal to the rod set Q2, the signal as being selected to excite different fragments, as detailed below, and the basic scheme is described in relation to the third fragmentation or mass selection step MS3 with the control unit 24.

Each ion has a secular frequency ω which is related to the drive frequency in the following equation.

$$\omega = \frac{q}{2\sqrt{2}} \times \Omega \qquad (1)$$

where q is a Mathieu parameter given by

$$q = \frac{4ev}{mr^2}\Omega^2 \qquad (2)$$

In accordance with the present invention, an excitation voltage is applied to the rod set Q2 at a frequency which is twice the secular frequency, i.e. with a frequency of $\omega=2\omega$ ion. This would be at a potential v, in the range of 1.5 to 40 volts. This potential will be added to each of the potentials supplied to each pair of rods of the rod set Q2. Thus, the potential supplied to the pairs of rods would be as follows:

$$V\cos\Omega t + v\cos(\omega t + \phi) \qquad (3)$$
$$-V\cos\Omega t - v\cos(\omega t + \phi)$$

where ϕ is simply a factor to allow for the fact that the two signals need not necessarily be in phase.

Thus, to effect the different steps of MS3 and MS4, it is a matter of selecting different frequencies of ω, corresponding to ions of interest, as explained in greater detail in relation to the examples below.

Additionally, an important aspect of the invention is to

provide a modulation to the additional excitation provided by the power supplies 24, 28. For this purpose, each power supply 24, 28 is shown with a respective modulation control unit 26, 30. For some purposes, it may be suitable or possible to provide a single modulation control unit.

Modulation control units 26, 30 effectively turn on and off the power supplies 24, 28, with a square wave signal at a frequency of, for example 2 Hz. In other words, the power supply 24, 28 as the case may be, would be turned on for .25 seconds, turned off for .25 seconds, etc. The reason for this is to provide data with and without excitation, to enable subtraction of the different signals obtained. In this context, the inventor has realized that comparing results with excitation on and excitation off for any lengthy time period is impractical, since any analyzer or detector tends to show drift for a variety of reasons. That is, a signal measured will drift by the order of a few per cent over time. In many cases, as detailed below, comparison of two signals, with excitation on and excitation off, amounts to obtaining a small difference between two relatively large signals. If either one of these has drifted significantly, then this can lead to a major error in the small, calculated difference.

Figure 1 also shows a modification to a conventional mass spectrometer apparatus, required by the present invention. Thus, the detector 20 is connected to a switch 32. The switch 32 is connected to and controlled by either one of the modulation control units 26, 30. The switch 32 has two outputs connected to separate data storage devices 34, 36. Thus, the data storage device 34 is for when there is no excitation and the data storage device 36 is for when excitation is provided.

Then, in use, when modulation is effected by either of the units 26, 30, and note that this is irrespective of any voltage set by the power supply 24, 28, the output from the detector 20 is switched by the unit 32 alternately between the two data storage devices 34, 36, in synchronism with the modulation. This enables collection of two sets of data, one when excitation is effected and one when excitation is not effected. As detailed below, this gives different spectra, which can be subtracted from one

another.

Reference will now be made to Figure 2. This shows an apparatus indicated generally by the reference 40. The apparatus 40 is similar to the apparatus 10, and for simplicity and brevity, like components are given the same reference numeral and the description of these components is not repeated. In brief, the apparatus 40 includes the first three quadrupole rod sets Q0, Q1 and Q2, and associated control and power supply elements.

However, here, to replace the final quadrupole Q3 on detector 20, there is provided a time-of-flight (TOF) mass analyzer 42. In known manner, the TOF analyzer of section 42 includes a gating region 44 and a detector 46. Thus, in use, ions pass into the gating region 44 and are gated or pulsed out to travel down the main body of the TOF 42, following a drift tube, until detected at a detector 46.

It will be appreciated that any suitable form of TOF could be provided. Thus, the TOF could comprise a reflectron or the like.

Reference will now be made to Figures 3-6 and also to Tables 1 and 2, which show mass spectra data collected in accordance with the present invention. All this data was collected on an apparatus using a TOF section, as in Figure 2.

Referring first to Figure 3a, there is shown a mass spectrum resulting from carrying out the first two MS steps, MS1 and MS2, on bosentan, a low mass chemical or drug, with a mass of 580. Thus, in Q1, the voltages are set to select bosentan, which is then accelerated into Q2 to fragment, to generate the spectrum shown in Figure 3a. As shown, this includes some residual amount of the original bosentan at mass 580 and other significant peaks of fragments at 508, close to mass 200 and others.

Figure 3b then shows a spectrum obtained by applying the third MS step, MS3, with a frequency set to excite an ion with an m/z 508. This is achieved by applying a 4.5 volt excitation signal at a frequency of 220 kHz. As indicated on Figure 3b, this effects MS/MS/MS.

Figure 3b also shows a subtracted spectrum. Thus, Figure 3 shows the spectrum obtained by effecting the triple MS technique, with the

spectrum of Figure 3a subtracted. Any negative quantities are shown as zero. For example, the peak for mass 508 will, clearly, be much less in Figure 3b, so the substraction of the spectrum of Figure 3a would give a negative value; in figure 3b, this is simply shown as zero.

This technique has the effect of subtracting any fragments that were present as a result of the initial two-step power scheme of Figure 3a. For example, Figure 3a shows a significant peak at a mass just above 200. This is still present in Figure 3b, but because of the subtraction that has taken place, one can be certain that this peak in Figure 3b is a result of fragmentation of the 508 ion, rather than the ion in the peak of Figure 3a simply carried over into Figure 3b without further fragmentation or alteration.

As shown in Figures 3c and 3d, similar to Figure 3b, but for masses 202 and 280. These again were achieved by effecting MS/MS/MS, and then subtraction of the spectra of Figure 3a. Thus, the spectra of Figures 3b, 3c and 3d clearly show the fragmentation spectra obtained by excitation of the selected fragment from the Figure 3a spectra, without any interference or contamination by fragments left over from the first fragmentation step.

Figure 3e shows a scan obtained by effecting modulation with modulation control unit 26, to provide the received signal into the two separate data streams, to collect two sets of data. However, the voltage supplied by the unit 24 is set to zero. In effect, Figure 3e shows the subtraction of what in theory should be two identical outputs. As can be seen, the spectra does show some measurable peaks. Note that these peaks result from, in effect, the subtraction of two relatively large quantities, to give a small difference. The vertical scale in Figure 3 is different from that in the other figures. What this shows is that there will, in practice, be some fluctuation of the signal, and this can be some measure of the fluctuation for individual fragments, and it can be noted that the fragment 202 shows a significant fluctuation.

Referring to Figures 4 and 4b these show, in greater detail, a

graphical representation of the signal obtained around the peak 124 and 122, as a result of exciting the fragment 202; thus these figures show details of the scan of Figure 3c.

Figure 4a shows two peaks 50 and 51. Peak 50 is the signal obtained with the additional excitation provided by the unit 24 turned off, and this also shows error bars indicating the variance in the signal obtained. Peak 51 shows the signal obtained with power supply 24 actuated, to provide excitation of fragment 202, generating an additional quantity of the ion around mass 124. A subtracted spectrum would effectively show peak 51 minus peak 50. This shows that a fragmentation of ion 202 does add significantly to a fragment at mass 124.

Figure 4b shows similar peaks 52 and 53 at mass 122. Again, error bars for the peak 52 are shown. Peak 52 shows the spectra with no excitation of ion 202, while peak 53 shows the spectra with 202 excited. This shows where the two peaks are effectively identical, allowing for a margin of error. In other words, fragmentation of ion 202 does not add significantly to the signal at mass 122.

This is explained further in relation to Tables 1 and 2. It is here noted that an important aspect of the present invention is a technique for determining when fragmentation of a particular ion has added to the signal for a smaller fragment, and when no such effect is present. This is based on two basic principles, namely: firstly, simply subtracting the two peaks, as indicated for the peaks in Figures 4a, 4b and determining that there is a significant additional added signal, when there is a significant and measurable difference between the two peaks; and comparing two peaks to determine if there is significant fluctuation in values, both positive and negative, close to a peak of interest. This latter feature is explained in greater detail in relation to Tables 1 and 2.

Referring first to Table 1, this shows four sets of data, for different peaks at, approximately 124, 98, 106 and 79, where it is determined that fragmentation of the 202 ion did add significantly to a peak. These peaks were chosen, representative of, respectively, medium, little, big and

little peaks. For each ion, there are two columns, indicating the count made, with excitation on and excitation off respectively.

Thus, for ion 124, counts are obtained at masses ranging from 124.0131 to 124.0735. On the right hand side, a column headed "Diff" indicates the differences between the on and off signals. One can note that, for all of these masses, except for the first one, there is some positive difference.

The final column calculates a significance factor T, or Sig. This is calculated by the following equation:

$$T = Sig = \frac{\text{detection signal, modulation on} - \text{detection signal, modulation off}}{\sqrt{\sigma^2 \text{ modulation on} + \sigma^2 \text{modulation off}}} \qquad (4)$$

where σ is the standard deviation. Here, a value of T of two or less, indicates that there is a greater than 5% probability that the excitation on and off signals are the same. On the other hand, for this mass 124, one can see that the values of T, at the peak, are in excess of 10, clearly indicative of a substantial difference, and this is borne out by the visual representation in Figure 4a.

Similar results, although not quite so strong, were obtained for the peak and mass 98. This again shows that, for nearly all values around the peak 98, the on signal gave a higher signal than the off signal. Again, value of T was quite high around the peak.

In general, it would be noted that it is more difficult to make a clear determination for smaller peaks.

For a large or big peak, as shown for the mass 106, the difference between the on and off signals was significant, and it is noted that the value of T reached a value of in excess of 57 close to the peak. This is clearly indicative of a substantial difference between the on and off signals, thereby indicating that the fragmentation of ion 202 did contribute significantly to the fragment and mass 106.

Finally, for the ion at mass 79, this represents another, smaller peak. This again gives a clear indication that there was a difference between the two signals.

TABLE 1

MASS	ON	OFF		DIFF	T
		MED#, YES			
124.0131	7	11	124.0131	0	0
124.0186	20	11	124.0186	9	1.61644772
124.0241	40	36	124.0241	8	0.91766294
124.0296	162	149	124.0296	16	0.90727676
124.0351	1117	874	124.0351	243	5.4459123
124.0406	3854	3036	124.04063	818	9.85470646
124.0461	6377	4865	124.0461	1524	14.3735213
124.0516	5321	4073	124.0516	1250	12.8968823
124.0571	2596	2164	124.0571	432	6.26152719
124.0626	1420	1163	124.0626	262	5.15512367
124.0681	1016	829	124.0681	193	4.4932349
124.0735	663	566	124.0735	115	3.28036296
		LITTLE,YES			
98.0192	1	1	98.0192	0	0
98.0241	4	2	98.0241	2	0.81649658

98.0289	13	13	98.0289	2	0.39223227
98.0338	61	28	98.0338	34	3.60399279
98.0387	91	66	98.0387	25	1.99521721
98.0436	103	51	98.0436	52	4.19027941
98.0485	43	33	98.0485	15	1.720618
98.0534	26	15	98.0534	12	1.87408514
98.0583	6	13	98.0583	1	0.22941573
98.0632	7	5	98.0632	5	1.44337567
98.068	1	6	98.068	1	0.37796447
98.0729	3	2	98.0729	3	1.34164079
98.0778	3	5	98.0778	0	0
98.0827	3	6	98.0827	0	0
		BIG, YES			
105.9971	18	11	105.9971	8	1.48556271
106.0021	10	7	106.0021	4	0.9701425
106.0072	29	11	106.0072	18	2.84604989
106.0123	46	19	106.0123	30	3.72104204
106.0174	120	58	106.0174	62	4.64709647
106.0225	803	437	106.0225	366	10.3937016
106.0275	5560	2858	106.0275	2702	29.4497006
106.0326	16232	8273	106.0326	7959	50.842998
106.0377	20957	10723	106.0377	10234	57.4980116

106.0428	13267	6652	106.0428	6615	46.8701219
106.0479	5185	2784	106.0479	2401	26.896158
106.053	2119	1174	106.053	945	16.4678136
106.058	1362	766	106.058	596	12.9199385
		V.LITTLE,YES			
79.0072	0	0	79.0072	0	#DIV/0!
79.0116	1	1	79.0116	1	0.70710678
79.016	8	2	79.016	7	2.21359436
79.0204	27	9	79.0204	19	3.16666667
79.0248	38	12	79.0248	26	3.67695526
79.0291	58	9	79.0291	49	5.98630277
79.0335	36	5	79.0335	31	4.84138662
79.0379	15	5	79.0379	11	2.45967478
79.0423	7	4	79.0423	4	1.20604538
79.0467	6	5	79.0467	3	0.90453403
79.0511	11	5	79.0511	6	1.5
79.0555	11	2	79.0555	9	2.49615088
79.0598	0	3	79.0598	0	0
79.0642	2	1	79.0642	2	1.15470054
79.0686	4	0	79.0686	4	2
79.073	3	0	79.073	3	1.73205081

Turning to Table 2, this shows sets of data indicating a situation, where fragmentation of ion 202 showed little variation in the on and off signals, indicating that the peaks were essentially the same, and for which the additional third MS step added nothing to the peak. Table 2 again shows, in the same order, data for a medium, little, big and little peaks, at masses 122, 131, 123 and 103 respectively.

For a first peak at 122, it can be noted that the difference column shows very small numbers, and many are negative; in this data representation, negative numbers are simply shown as zero.

The column for the factor T shows that for the mass 122, T often has a value of much less than 1, and only exceeds 1 for a couple of the data points. This is clearly indicative of two peaks that are the same and have no statistically different magnitude.

There is a similar effect for a small or little peak for the mass 131. Here, the values of T are even smaller, and it can be seen that many of the values for the difference figure are negative or very small.

For a big peak at mass 123, due to the larger size of the peaks, values for the difference and significance parameter T are larger Here, a review of the various values of the parameter T again clearly shows that these two peaks are substantially the same.

Finally, for mass 103, it can be noted that the values for the difference in T data are all extremely small. Again, a clear indication that there is no statistically significant difference between the two peaks.

TABLE 2

MASS	ON	OFF		DIFF	T
	MED#,NO				
122.0154	12	9	122.0154	3	0.65465367
122.0208	27	31	122.0208	0	0

122.0263	76	92	122.0263	3	0.23145502
122.0318	170	162	122.0318	16	0.87811408
122.0372	153	159	122.0372	5	0.28306926
122.0427	364	411	122.0427	1	0.03592106
122.0481	1192	1319	122.0481	10	0.19956145
122.0536	2480	2365	122.0536	123	1.76708818
122.059	2381	2496	122.059	4	0.05727744
122.0645	1325	1401	122.0645	0	0
122.0699	622	596	122.0699	26	0.74498873
122.0754	285	257	122.0754	40	1.71814712
122.0808	159	170	122.0808	4	0.22052714
	LITTLE,NO				
131.017	1	2	131.017	0	0
131.0226	12	12	131.0226	1	.020412415
131.0282	18	20	131.0282	3	0.48666426
131.0339	26	22	131.0339	7	1.01036297
131.0395	32	49	131.0395	0	0
131.0452	132	133	131.0452	11	0.67572463
131.0508	324	311	131.0508	16	0.63494063
131.0565	463	507	131.0565	11	0.35318871
131.0621	335	333	131.0621	15	0.58036742
131.0678	172	186	131.0678	17	0.89847792

131.0734	212	226	131.0734	0	0
131.0791	385	386	131.0791	11	0.39615532
131.0847	405	391	131.0847	31	1.09876587
131.0904	204	203	131.0904	8	0.39654528
131.096	81	86	131.096	3	0.23214697
		BIG,NO			
123.0259	220	263	123.0259	3	0.13650473
123.0314	1108	1098	123.0314	39	0.83035127
123.0368	2737	2943	123.0368	33	0.43786454
123.0423	3539	3554	123.0423	85	1.00926206
123.0478	2622	2738	213.0478	19	0.25952022
123.0533	3409	3343	123.0533	123	1.49688658
123.0587	7021	7081	213.0587	62	0.52209716
123.0642	8916	8623	123.0642	313	2.36342554
123.0697	5861	5698	23.0697	163	1.51609869
123.0752	2345	2247	23.0752	107	1.57900257
123.0806	957	945	123.0806	53	1.21526395
123.0861	585	587	123.0861	33	0.96394029
	V.LITTLE,NO				
103.0308	4	9	103.0308	0	0

103.0358	10	10	103.0358	6	1.34164079
103.0408	38	37	103.0408	6	0.69282032
103.0458	79	85	103.0458	0	0
103.0508	140	146	103.0508	6	0.35478744
103.0558	103	112	103.0558	6	0.4091966
103.0608	46	47	103.0608	4	0.41478068
103.0658	8	22	103.0658	0	0
103.0708	14	15	103.0708	0	0
103.0758	6	3	103.0758	3	1
103.0809	2	2	103.0809	1	0.5
103.0859	4	2	103.0859	2	0.81649658
103.0909	5	4	103.0909	2	0.66666667
103.0959	2	3	103.0959	0	0
103.1009	2	4	103.1009	0	0
103.1059	0	0	103.1059	0	#DIV/0!

Turning to Figure 5, this shows test results and spectra obtained for the drug taxol. Figure 5a again shows the basic two-step MS/MS process. That is, taxol was selected in Q1, for transmission into Q2; the taxol is then accelerated into Q2 with a suitable potential difference, to cause CID or fragmentation of the taxol in Q2. The spectra in Figure 5a was then obtained.

Figure 5b then shows the spectrum obtained by further excitation, i.e. the third MS step, i.e. MS3, of the ion link. Figure 5b again is a subtracted spectrum, with the spectrum of Figure 5a subtracted from the spectrum obtained with the mass excited. This shows a significant range of fragments for approximately 100 m/z to 400 m/z. Notably, even though

there are significant peaks in this range in Figure 5a, the same ions are also generated by the subsequent fragmentation.

Figure 5c again shows a subtraction spectrum obtained without any excitation. In other words, with modulation unit 26 actuated, to cause the data to be divided into two sets of data, but with the power supply 24, set to give zero excitation. Surprisingly, for taxol, this shows a significant residual background.

Referring now to Figures 6a, 6b and 6c, these show further spectra obtained for reserpine. Figure 6a again shows just the first two MS steps, where reserpine is selected in Q1, accelerated and fragmented in Q2. Additionally, here Figure 6a just shows the low mass end of the fragment spectrum up to approximately mass 200. This shows that reserpine with an m/z of 609 generates significant fragments at 174.1 and 195.1.

Figure 6b then shows the spectrum obtain by a third MS step, where the fragment at 174 was excited. As might be expected, this shows a much reduced peak for the mass 174, and an increase in the number and intensity of fragments below mass 174, notably peaks at 130.1 and 131.1 Unlike earlier figures, Figure 6b is an unsubtracted spectrum.

If the spectrum of Figure 6a is subtracted from Figure 6b, the spectra of Figure 6c is obtained. Note that this is on a different scale. This clearly shows a significant reduction in the peak at 195.1, as this was present in the original spectrum of Figure 6a.This spectrum also emphasizes the contribution made to the various other fragments by the third MS step, the major peaks being identified in Figure 6c.

Reference will now be made to Figures 7a, 7b and 7c. Figure 7a shows part of the spectrum of Figure 6a but only up to a mass of approximately 190. This enables a different scale to be used, to emphasize the size of the different peaks.

Figure 7b then shows a spectrum obtained for a four-step excitation scheme. Here, the fourth MS step, MS4 was effected utilizing the power supply 28 and modulation unit 30. For this scheme, the excitation as a third MS step, by the power supply 24, is continuous, without any

modulation by the unit 26. The spectrum obtained is then subject to further excitation of the mass at 130/131; these two masses are so close together, that it is impossible to obtain excitation of just one mass. Again, Figure 7b is an unsubtracted spectrum.

Figure 7c then shows the spectrum of Figure 7b, with that of Figure 7a subtracted. This again, shows elimination of peaks due to previous fragmentation and hence solely the peaks resulting from ions generated by fragmentation of the ions of mass 130, 131. It should be noted that for the fourth step MS/MS/MS/MS procedure, excitation from the two power supplies 24, 28 is provided simultaneously. As noted, the power supply 24 is unmodulated, i.e. continuous, while the excitation from power supply 28 is modulated at a modulation of, for example, 2 Hz.

Reference will now be made to Figures 8a-8d, which show a series of spectra, indicating the effects of varying the excitation voltage. Figure 8a again corresponds to Figure 6a, and shows the fragment spectrum obtained from the initial fragmentation of the Reserpine, again showing significant peaks at 174.1 and 195.1. In this case, the larger peak at 195.1 was selected for further excitation. This was excited at a frequency of ?, and at different voltages of 1.5, 2.5 and 3.5, to obtain the spectra of Figures 8b, 8c and 8d. Each of these spectra 8b-8d are subtracted spectra, that is the spectra obtained with the excitation and subsequent subtraction of the spectrum of Figure 8a. They are also unfiltered.

As might be expected, the peak at 195 is largely eliminated as a result of the excitation. It can be noted that at low excitation potentials, a peak is shown with an ion close to mass 190, and this peak reduces significantly, as the excitation voltage is increased. Correspondingly, peaks with smaller fragment ions increase. This is to be expected.

It will be appreciated that, while the invention has been described as effected with a quadrupole, it can be carried out in any suitable collision cell, and in particular any collision cell where quadrupolar fields can be applied. Thus it could also be carried in a magnetic sector instrument, as one example.

CLAIMS:

1. A method of analyzing a substance, the method comprising the steps of:

(1) creating a stream of ions in said substance;

(2) supplying the ions to a collision cell including a quadrupole rod set for guiding the ions and a buffer gas;

(3) applying an RF voltage to the quadrupole rod set to guide ions through the quadrupole rod set;

(4) supplying an additional alternating current signal to the quadrupole rod set at a frequency selected to cause resonance excitation of the secular frequency of a desired ion, whereby said desired ions are excited and undergo collision with the buffer gas causing fragmentation;

(5) modulating the alternating current signal applied in step (4) whereby periods in which said alternating current signal is applied alternate with periods in which said alternating signal is not applied;

(6) analyzing the ion spectrum after fragmentation and collecting one set of data for one spectrum, representative of the ion spectrum when the alternating current signal is applied and a another set of data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied.

2. A method as claimed in claim 1, wherein the alternating current signal applied is at a frequency which is twice the secular frequency of the desired ion.

3. A method as claimed in claim 1, which includes passing the stream of ions through a first mass analyzer to select a precursor ion of interest, and passing the precursor ion into the collision cell.

4. A method as claimed in claim 3, which includes providing a potential difference between the first mass analyzer and the collision cell, to accelerate the precursor ion into the collision cell, whereby the precursor ions gain sufficient velocity to collide with the buffer gas to cause fragmentation, and wherein step (4) comprises applying an alternating current signal to excite a fragment of the precursor ion, said fragment comprising the desired ion.

5. A method as claimed in claim 3 or 4, which includes applying a second alternating current signal to the quadrupole rod set, to excite a fragment ion resulting from resonance excitation of said desired ion, thereby to generate secondary fragment ions and wherein step (5) comprises modulating the second alternating current signal.

6. A method as claimed in claim 1, 2, or 4, which includes subtracting said one spectrum from the other spectrum to obtain a subtracted spectrum.

7. A method as claimed in claim 5, which includes subtracting said one spectrum from said other spectrum to obtain a subtracted spectrum.

8. An apparatus, for analyzing a substance by resonance excitation of selected ions and selective collision-induced dissociation, the apparatus comprising:

an ion source for generating a stream of ions;

a collision cell, including a quadrupole ion guide, for receiving a stream of precursor ions and provided with a buffer gas, for collision-induced dissociation between the parent ions and the buffer gas;

a power supply connected to the quadrupole rod set for generating an RF field in the quadrupole rod set for guiding ions and for applying an additional alternating current field at a frequency selected to

excite a desired ion;

a modulation means connected to the power supply, for modulating the alternating current signal, whereby periods in which said alternating current signal are applied alternate with periods in which the alternating current signal is not applied.

9. An apparatus as claimed in claim 8, which additionally includes a detector for detecting fragment ions exiting the collision cell, a switch connected to the detector, two data storage devices connected to the switch, and a connection between the modulation control unit and the switch, whereby the switch switches detected data for periods when the alternating current signal is applied to one data storage device and collected data for periods when the alternating current signal is not applied to the other storage device.

10. An apparatus as claimed in claim 9, which includes a second power supply connected to the quadrupole rod set, a second modulation unit connected to the second power supply and also to the switch, before applying a second alternating current signal, for excitation of a second ion.

11. An apparatus as claimed in claim 10, which includes a first mass analysis section for selecting a parent ion.

12. An apparatus as claimed in claim 11, which includes a final mass analysis section, including the detector, for analyzing fragment ions from the collision cell.

13. An apparatus as claimed in claim 12, wherein the final mass analysis section comprises one of:

a scanning mass analyzer and a detector; and

a time-of-flight device, including the detector for

providing a small spectrum.

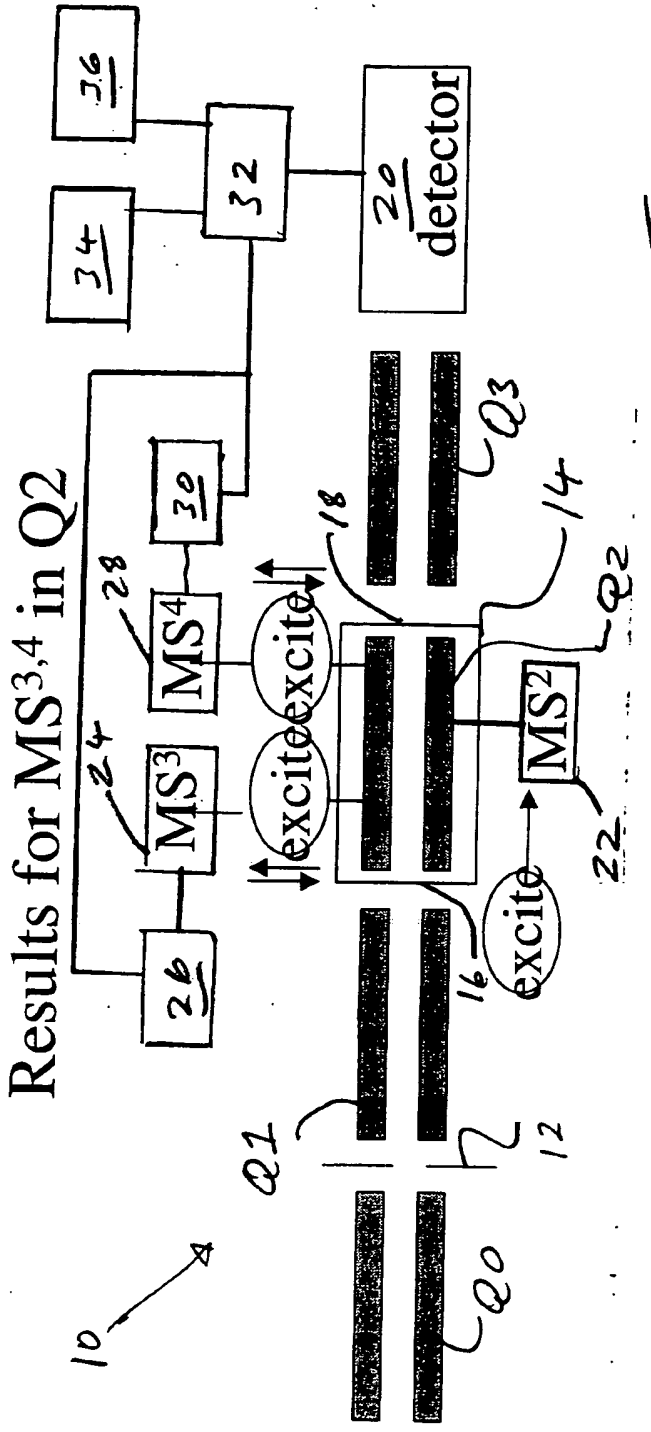

Results for MS3,4 in Q2
10
Q0
Q1
12
16
excite
22
Q2
MS2
14
Q3
18
excite
excite
24
MS3
28
MS4
30
26
32
34
36
20
detector

Fig 1

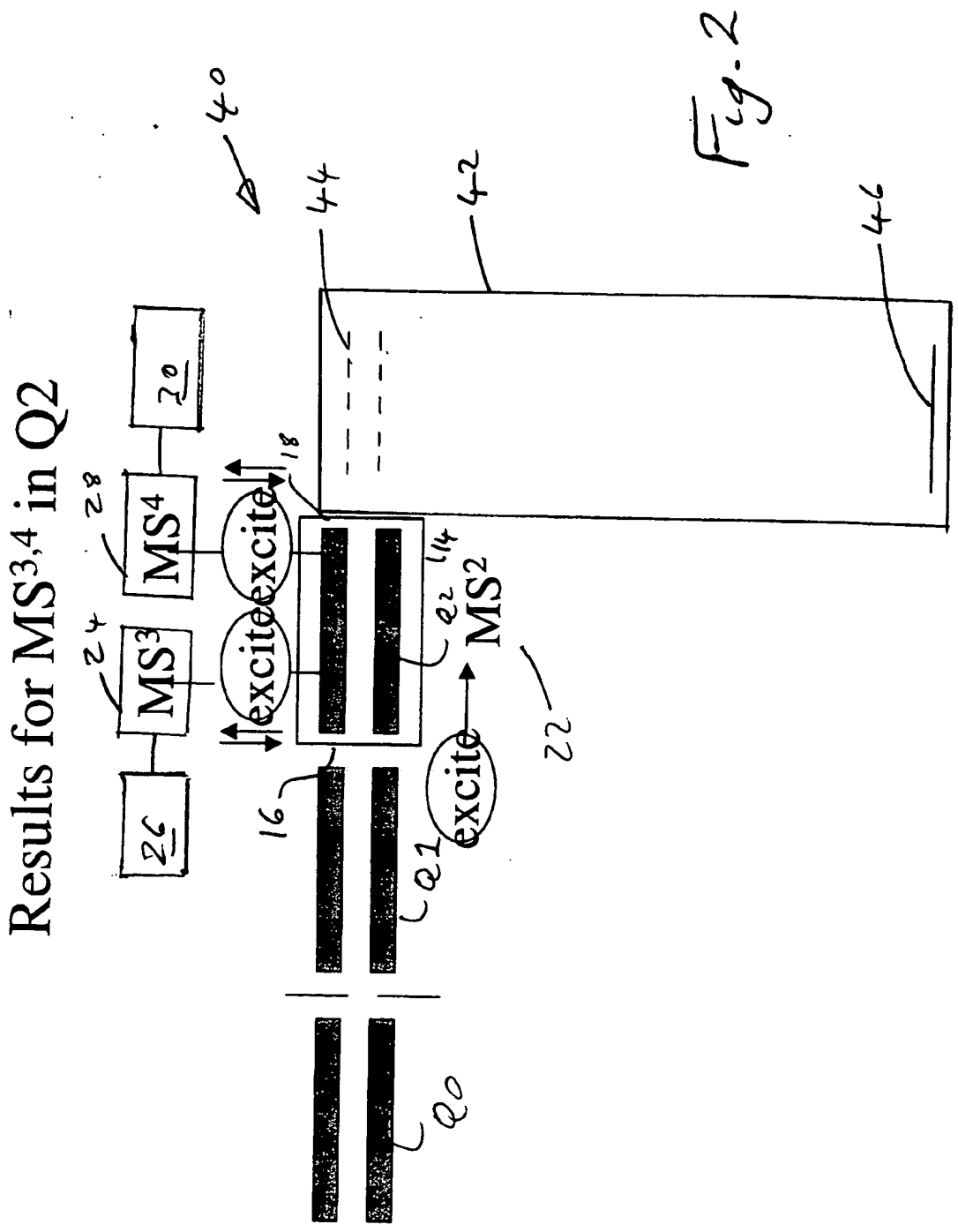

Results for MS3,4 in Q2
40
44
42
46
20
28
MS4
18
excite
24
MS3
excite
Q2
114
MS2
22
26
16
excite
Q1
Q0

Fig. 2

BioMultiView 1.5b3 Friday, May 21, 1999 5:42 PM

TOF Binary data from:"508-4.5V OFF"

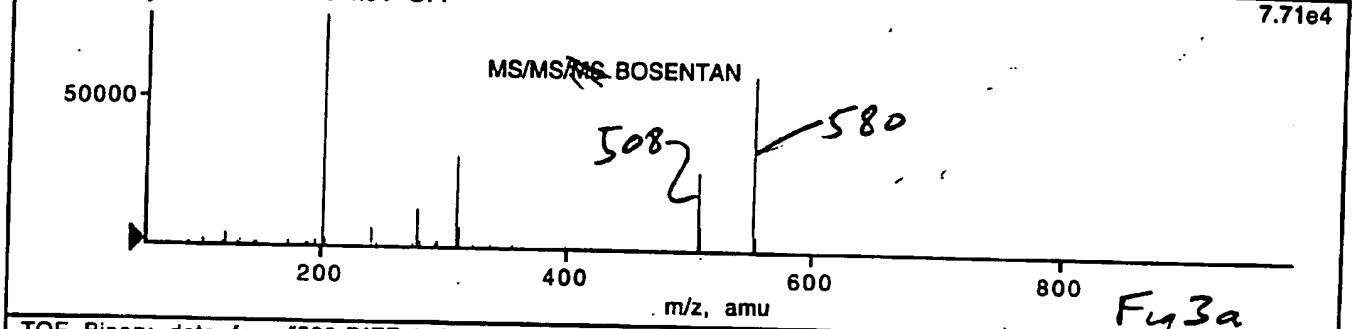


Fig 3a

TOF Binary data from:"508-DIFF-4.5V-220KHZ"

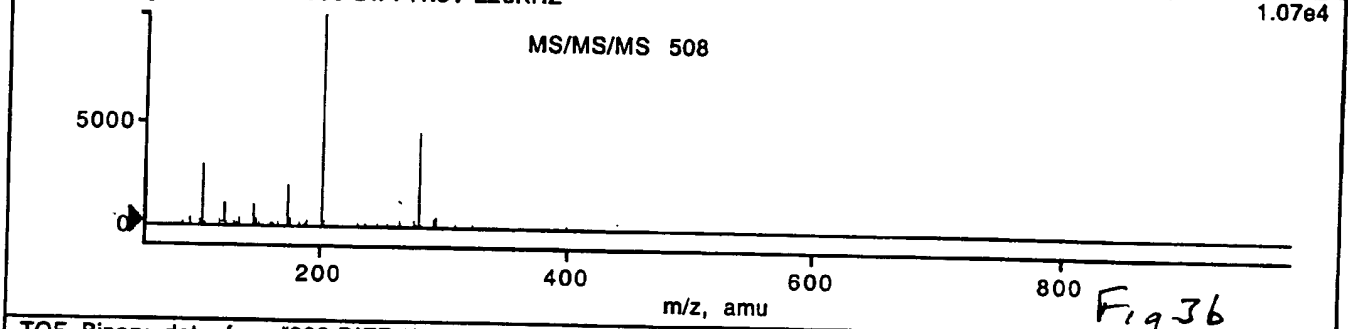


Fig 3b

TOF Binary data from:"202-DIFF-3V-585 KHZ"

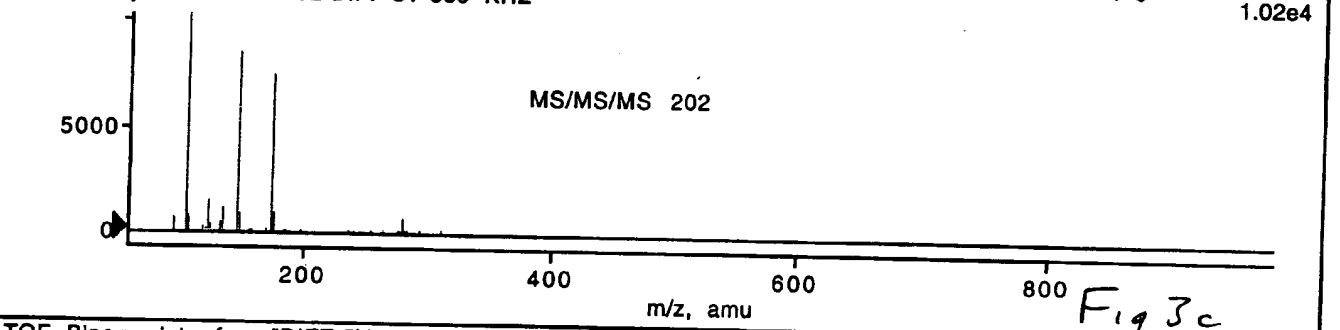


Fig 3c

TOF Binary data from:"DIFF-5V-280--80 SCANS-#3"

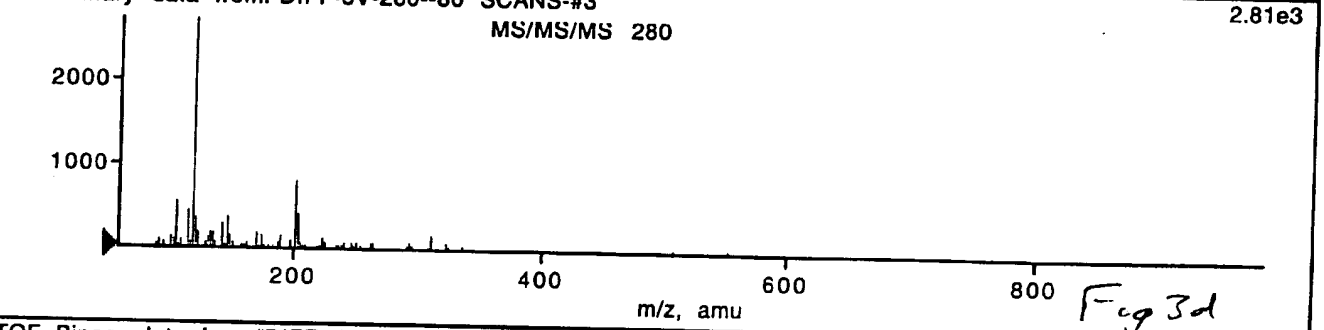


Fig 3d

TOF Binary data from:"DIFF-0V-280--80 SCANS-BACK"

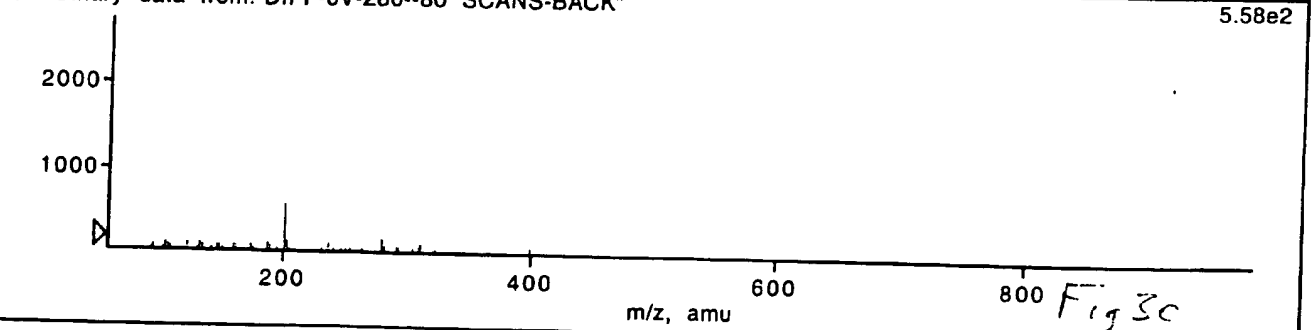
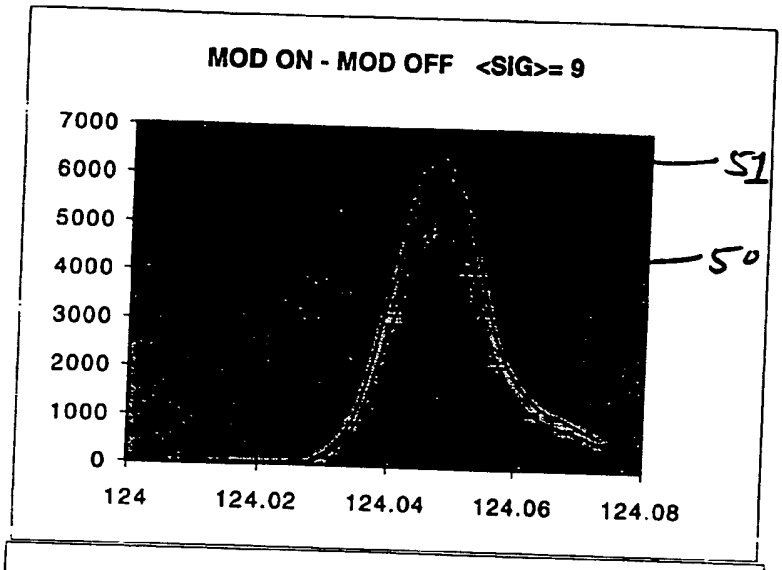


Fig 3e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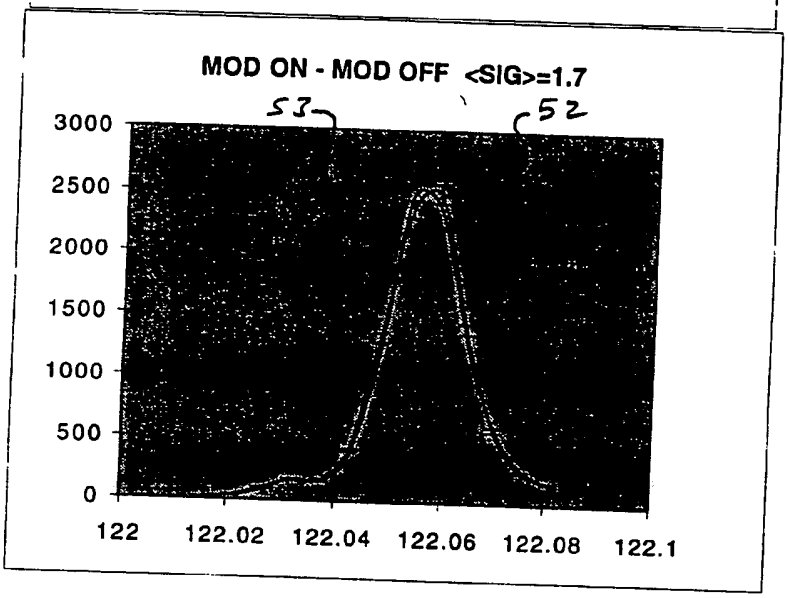
7000
6000
5000
4000
3000
2000
1000
0
124
124.02
124.04
124.06
124.08
51
50

Fig 4a


MOD ON - MOD OFF <SIG>=1.7
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52
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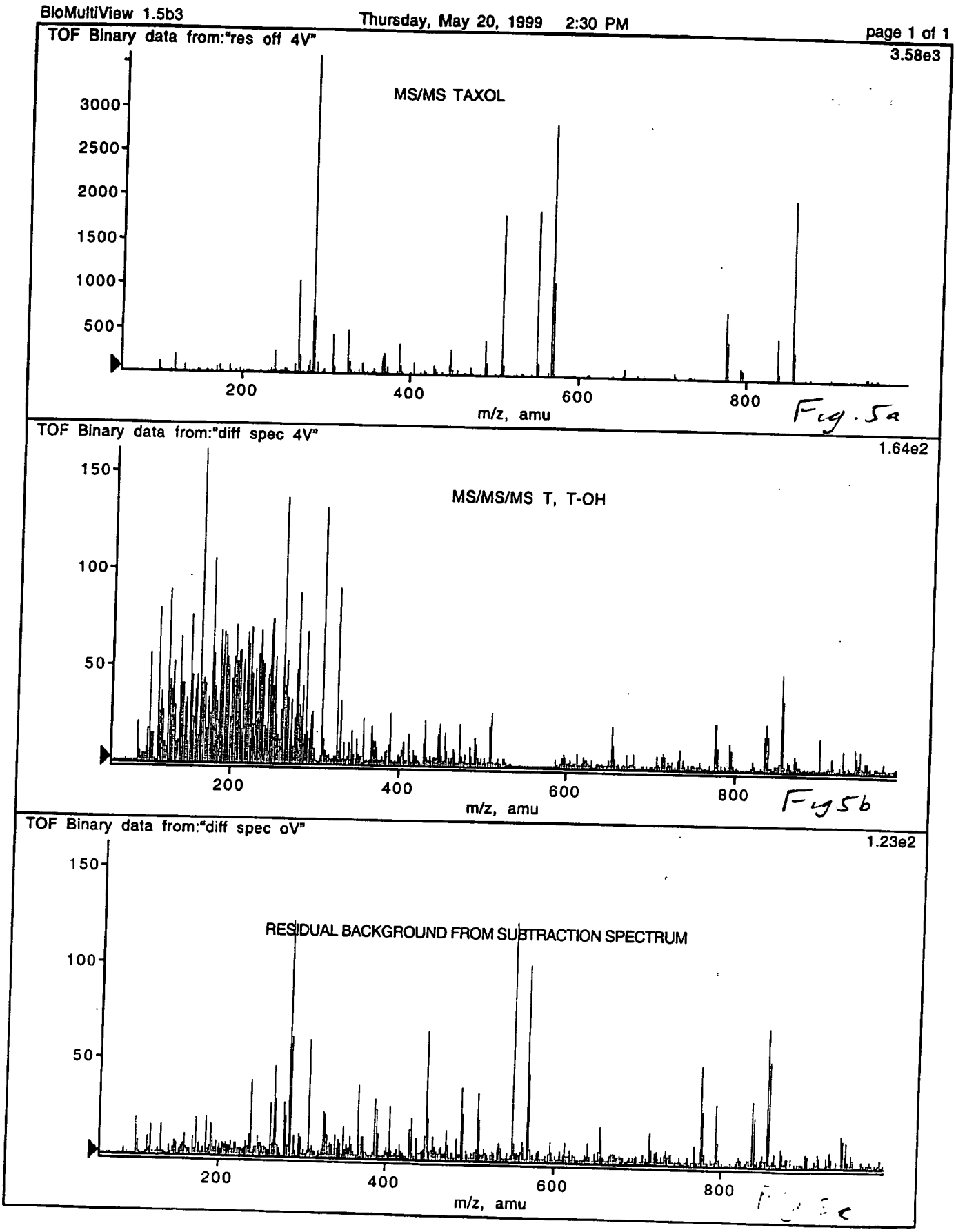
2500
2000
1500
1000
500
0
122
122.02
122.04
122.06
122.08
122.1

Fig 4b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Thursday, May 20, 1999 2:30 PM
page 1 of 1
TOF Binary data from:"res off 4V"
3.58e3
MS/MS TAXOL
3000
2500
2000
1500
1000
500
200
400
600
800
m/z, amu
Fig. 5a
TOF Binary data from:"diff spec 4V"
1.64e2
MS/MS/MS T, T-OH
150
100
50
200
400
600
800
m/z, amu
Fig 5b
TOF Binary data from:"diff spec oV"
1.23e2
RESIDUAL BACKGROUND FROM SUBTRACTION SPECTRUM
150
100
50
200
400
600
800
m/z, amu

OF Binary data from:"CAD 609 no excitation"

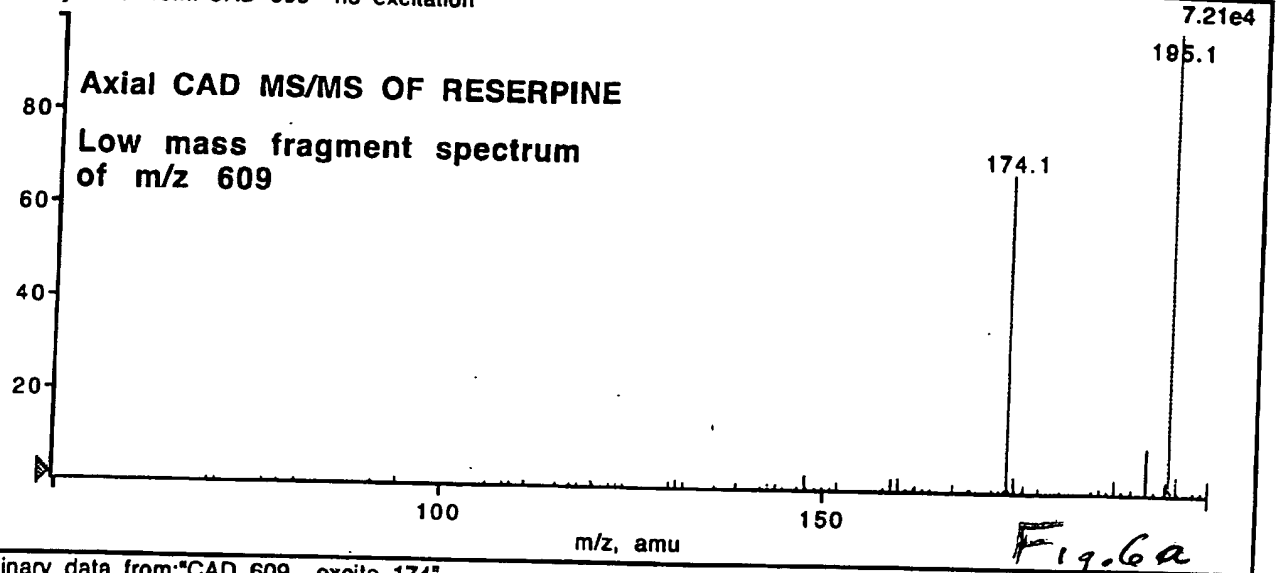


Fig.6a

F Binary data from:"CAD 609 excite 174"

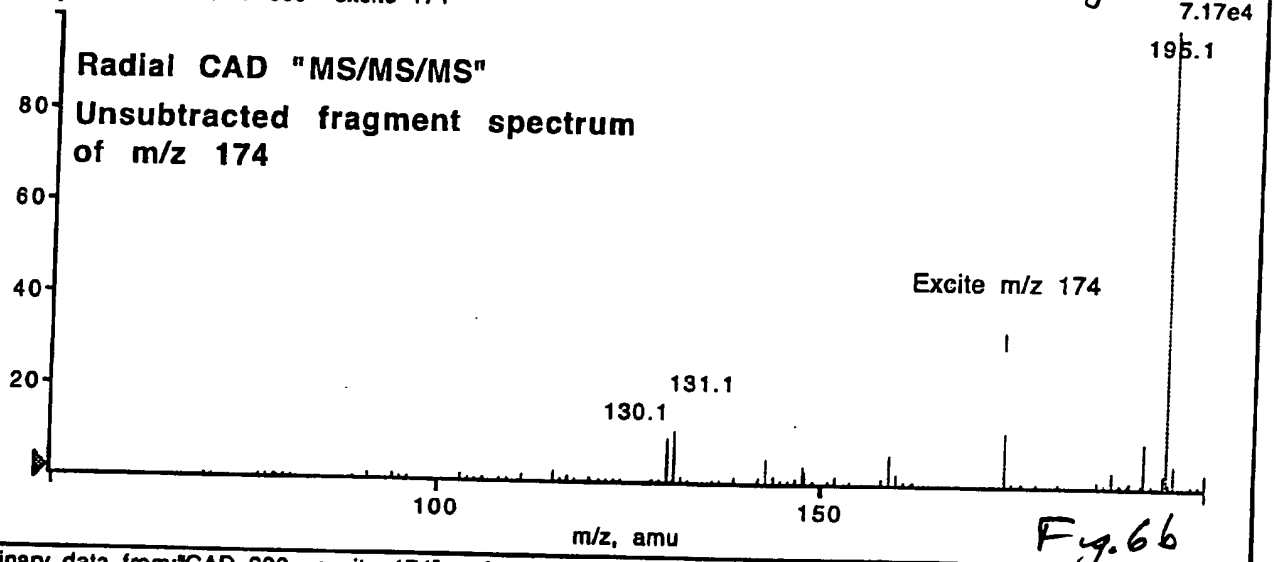


Fig.6b

Binary data from:"CAD 609 excite 174", subtracted

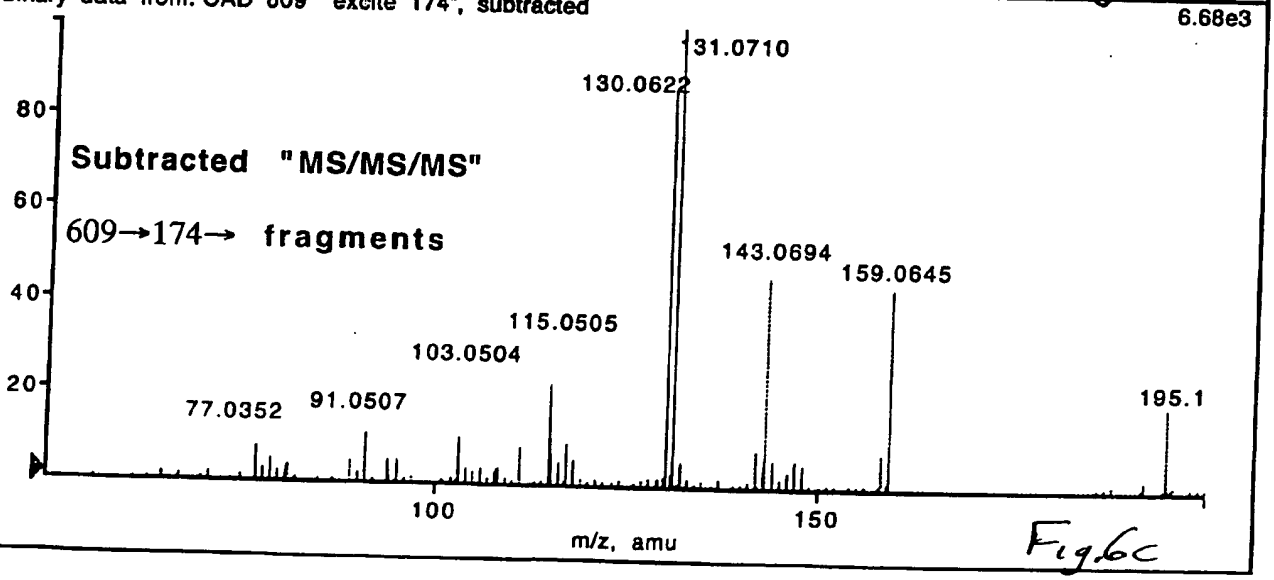


Fig.6c

Binary data from:"CAD 609, excite 174 only"

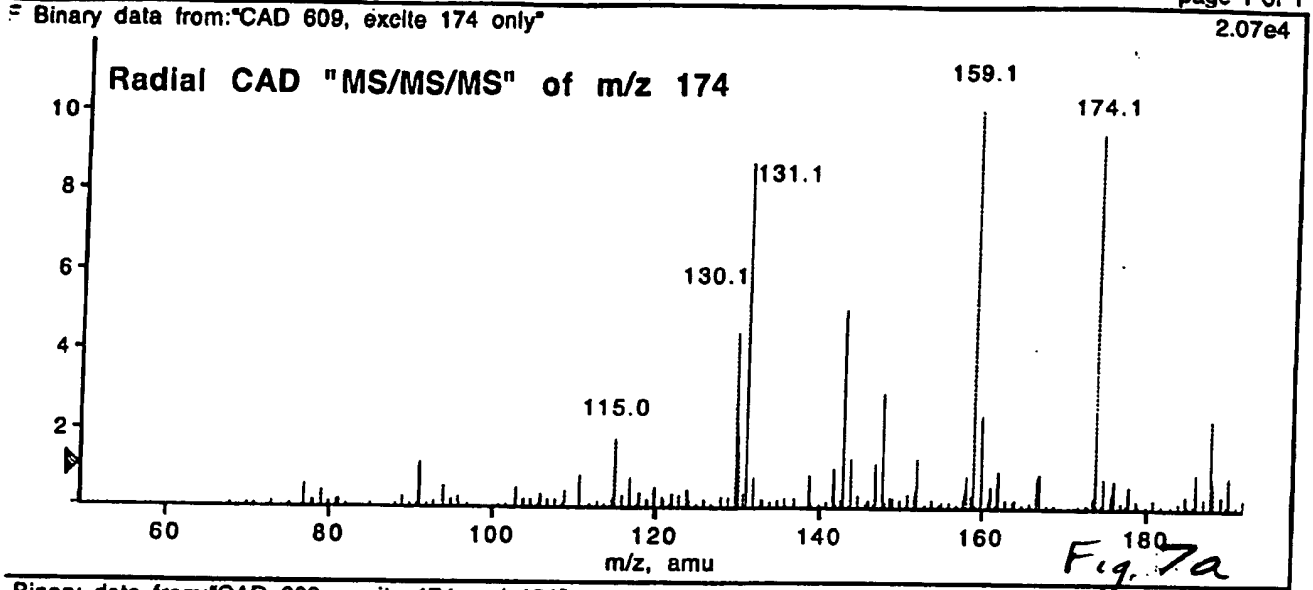


Fig. 7a

Binary data from:"CAD 609, excite 174 and 131"

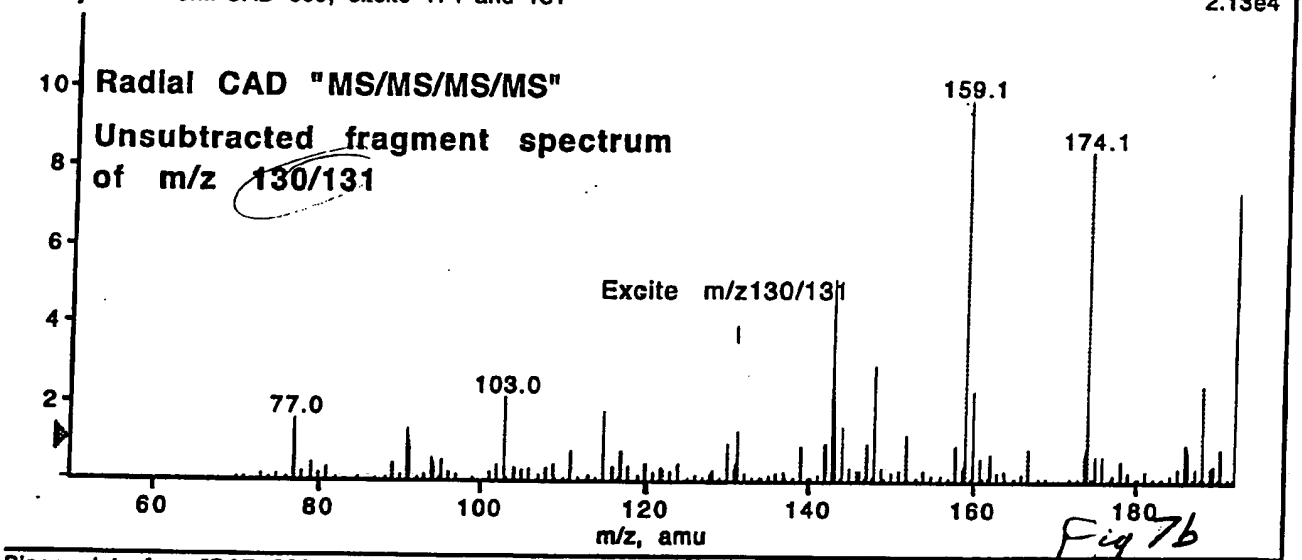


Fig 7b

Binary data from:"CAD 609, excite 174 and 131", subtracted

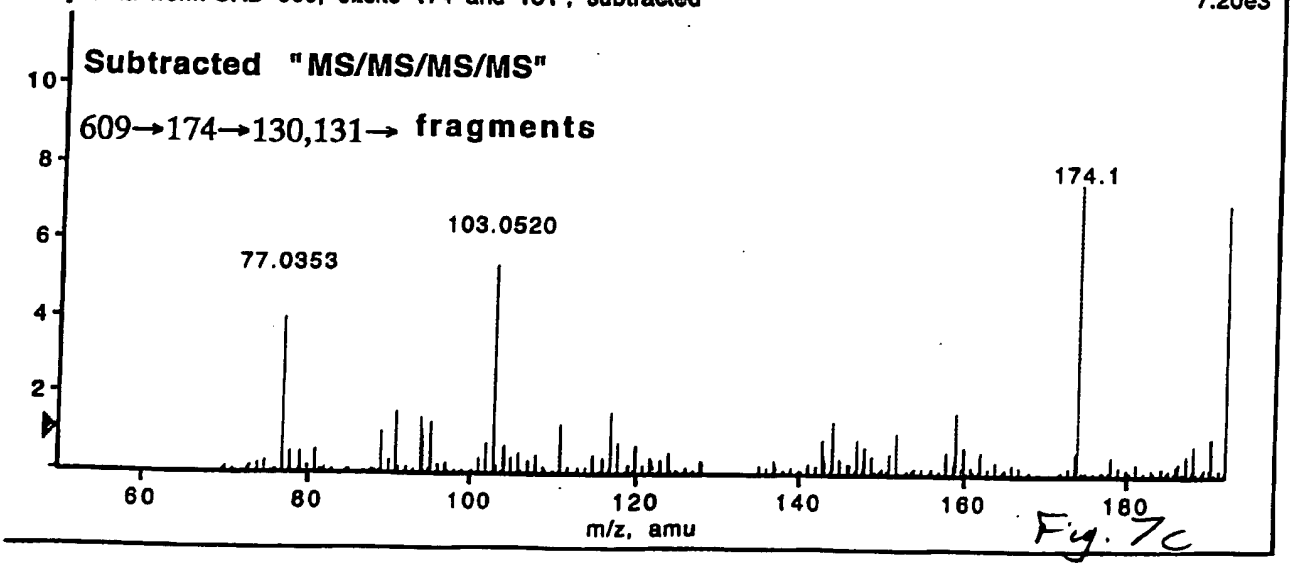
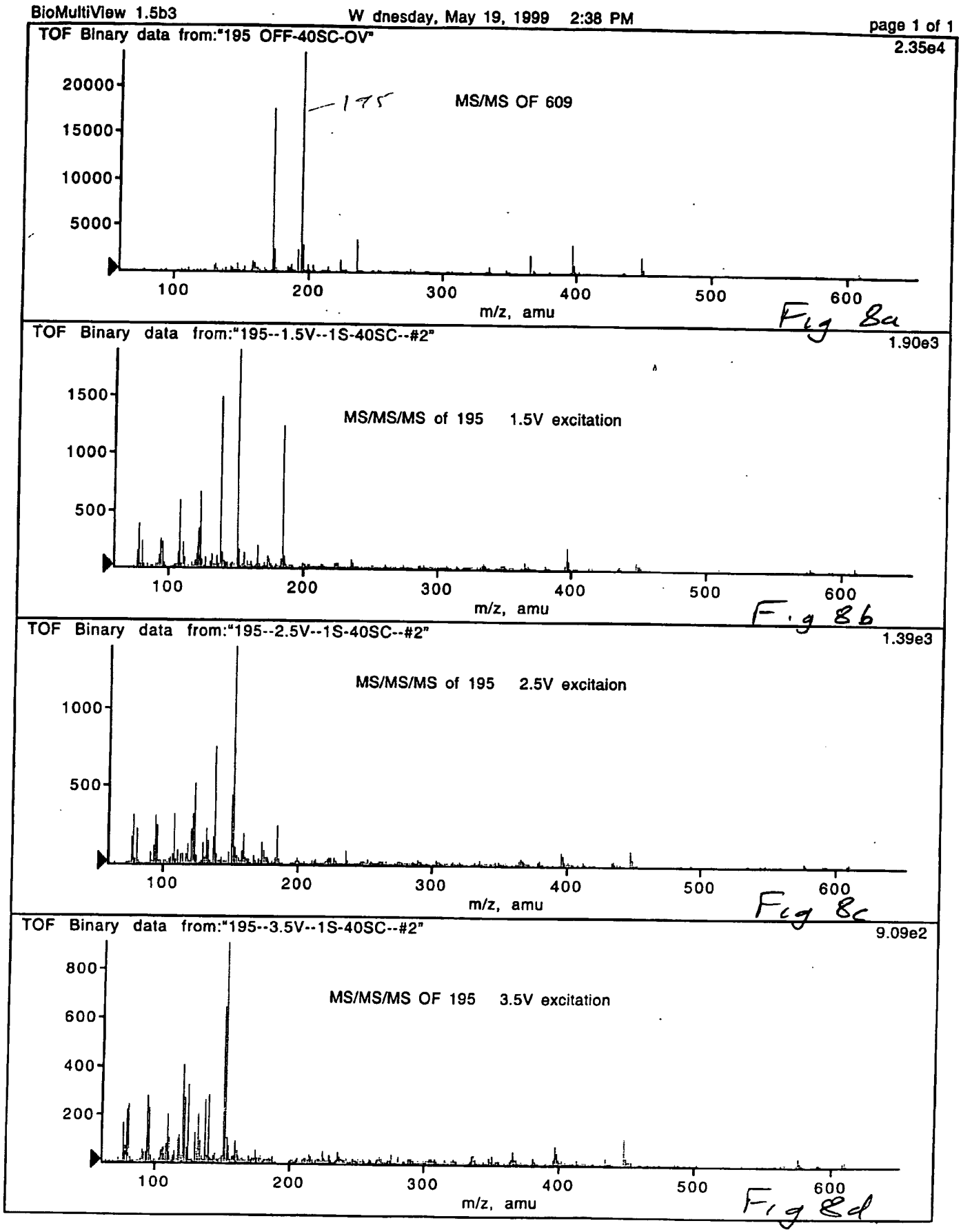


Fig. 7c


BioMultiView 1.5b3
W dnesday, May 19, 1999 2:38 PM
page 1 of 1
TOF Binary data from:"195 OFF-40SC-OV"
2.35e4
20000
15000
10000
5000
195
MS/MS OF 609
100
200
300
400
500
600
m/z, amu
Fig 8a
TOF Binary data from:"195--1.5V--1S-40SC--#2"
1.90e3
1500
1000
500
MS/MS/MS of 195 1.5V excitation
100
200
300
400
500
600
m/z, amu
Fig 8b
TOF Binary data from:"195--2.5V--1S-40SC--#2"
1.39e3
1000
500
MS/MS/MS of 195 2.5V excitaion
100
200
300
400
500
600
m/z, amu
Fig 8c
TOF Binary data from:"195--3.5V--1S-40SC--#2"
9.09e2
800
600
400
200
MS/MS/MS OF 195 3.5V excitation
100
200
300
400
500
600
m/z, amu
Fig 8d